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PHOTOPERIOD AND TEMPERATURE  
IN PHENOTYPE DETERMINATION OF PACIFIC SLOPE  
PIERINI: BIOSYSTEMATIC IMPLICATIONS<sup>1</sup>

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FOR THE PAST DOZEN YEARS I have been studying seasonal phenotype determination in the family Pieridae. As you all know, many butterflies and moths which have several generations a year display seasonal "forms" or phenotypes, often so different in appearance that they were described as different species; their conspecificity was proven only by breeding them, one from the other, or preferably by split-brood experiments in which different rearing conditions elicited different phenotypes in the progeny of single females. From the earliest days of evolutionary biology down almost to the present, such seasonal variation was subsumed by the familiar Victorian word "polymorphism." In the past decade, however, particularly since the advent of "electrophoretic genetics" — in which allelic frequencies are estimated by examining the mobility of enzymes in an electric field for a suitably large sample of the population in question — the word "polymorphism" has been increasingly appropriated by geneticists to refer specifically to variation which has a *genetic*, rather than an *environmental*, basis. This restriction can be traced to E. B. Ford's now classic definition of polymorphism as the occurrence in a population of more than one allele at a locus, such that the rarest allele is too common to be maintained solely by recurrent mutation. What, then, are we to call seasonal phenotypic variation which is under en-

<sup>1</sup> Read by John H. Lane at the 23rd annual meeting of the Pacific Slope section of the Lepidopterists' Society, Nevada State Museum, Carson City, Nevada, July 2, 1976.

vironmental control? We can call it just that; or we can use Ernst Mayr's convenient word *polyphenism*, coined in parallel to polymorphism, and defined as "the occurrence of several phenotypes in a population, the differences between which are not the result of genetic differences." Studies in various laboratories — H. J. Muller's and Rolf Reinhardt's in East Germany, Ward Watt's at Stanford, and our own — are establishing how widespread and complex a phenomenon seasonal polyphenism is in the Lepidoptera. We are finding, much as the great developmental geneticist C. H. Waddington foresaw twenty-some years ago, that developmental plasticity provides an alternative to genetic polymorphism which allows natural populations to cope with a variable environment in an economical, adaptive way. We are also finding that studies of the physiological mechanisms determining phenotype allow us to interpret the history of a specific group's climatic and geographic adaptation — and this in turn gives us clues bearing on the perennial problems of species diversity and community stability.

Mayr pointed out in 1961 that any adaptation can be studied from the standpoint of *proximate* or *ultimate* causality. Any time we ask a "why" question in biology, we are really asking several questions at different levels all at once. Let us consider the familiar Gray-Veined White, *Pieris napi* (*sens. lat.* — if you buy B. C. S. Warren's single-character taxonomy you can leave the room now). Everyone knows, or thinks he knows, that Californian *napi* are seasonally diphenic — they have a spring phenotype with black veins, known as *venosa*, and a summer one without black veins, called *castoria*. I'll show in a minute or three that you don't really know that, but first let's ask "Why does *Pieris napi* have two seasonal phenotypes?" There are several possible answers. At the proximate, or immediate, level we have physiological mechanisms which translate from some environmental cue to an eventual phenotype by affecting melanin pigment synthesis and deposition. The most complete studies of such systems have been done not on *Pieris napi* but on the Nymphalid *Polygonia c-aureum* in Japan by Hidaka, Aida, Fukuda, Endo, and Takahashi. Their papers, beginning 13 years ago, are not well known in this country; most English-speakers are still laboring under the misapprehension fostered by E. B. Ford in a casual remark in *Butterflies*, that the seasonal forms of *Polygonia* are food-determined. Actually *P. c-aureum* is broadly representative of seasonally polyphenic butterflies in

that the primary factor controlling adult phenotype is larval exposure to daylength (photoperiod), with some input from temperature. That shouldn't be too surprising. In middle latitudes photoperiod is a much more reliable predictor of season than temperature is: the daylength is essentially the same on every June 25, but the temperature may be wildly different. These Japanese workers have shown with painstaking thoroughness that long days interacting with high temperatures stimulate certain cells in the larval brain to produce a hormone, which travels through the nerves to the corpora cardiaca of the pupa, whence it is released into the hemolymph (blood) where it induces development of the summer adult phenotype. But that is not the end of the story: the dark, summer animals breed immediately, but light, winter animals are quite uninterested in sex — and Endo wanted to know why. It turns out that ovarian maturation and female pheromone (sex-stimulant chemical) production are also under neurosecretory control — the relevant hormone being daylength-dependent. In *Pieris napi*, unlike *Polygonia* species, there is no adult hibernation and sexual behavior is not at issue. But the hormonal regulation of adult phenotype is probably very similar.

We have now studied about a dozen *napi* populations from the Yukon to central New Mexico, including a variety of Californian ones. It appears that adult phenotype in the Gray-Veined White is under the control of photoperiod, temperature, and certain genes, interacting in complex — and immensely interesting — ways. The basic situation in nature is that pupae which go into programmed dormancy — “diapause” as it is known to physiologists — produce dark-veined, i.e. spring phenotypes, and those which develop directly, without diapause, produce light-veined, or summer ones. This applies even to populations which are normally single-brooded in nature, with “obligate” diapause — we can prevent diapause by appropriate rearing conditions, which lie outside the range of real environments the animals would encounter afield. Since the primary control of diapause is photoperiodic, until quite recently it was assumed that phenotype was physiologically coupled to development — that the hormonal control of diapause also affected pigment synthesis and deposition. But I now no longer believe that — at least, not quite. The blame for my disillusionment lies with August Weismann.

You may remember Weismann's name from your genetics classes; he first enunciated the dogma of the isolation of the germ cells from environmental influences — the critical repudiation of Lamarckian heredity, the inheritance of acquired characteristics. He was one of the greatest of the early Darwinians, and until his failing eyesight forced him to become a theoretician, he worked on Leps, including *Pieris napi*. Now, Weismann was a monstrous clever fellow: 70 years before Mayr he talked about multiple levels of causality, and he even discussed seasonal polyphenism from that standpoint. When he bred *napi*, biological photoperiodism hadn't been thought of yet; the influence of daylength on insect development remained to be discovered in the 1940s by Danilyevskiy in Russia. So Weismann figured temperature was *the* environmental cue, and designed experiments accordingly; and he got ambiguous results, at least with Pierids. Since we now know these critters to be strongly photoperiodic, I felt it necessary to repeat Weismann's experiments under controlled daylengths to determine whether there really might be a temperature effect. Not having central European *napi*, I settled for coastal central Californian.

It turned out that certain chilling treatment applied to *non-diapause* pupae quite unambiguously produced the spring phenotype! The identical treatment was much less effective on Inner Coast Range material. This difference among stocks underscores the rather obvious fact that physiological mechanisms have a genetic basis — the interface between Mayr's proximate and ultimate levels of causality. But how is one to account for the apparent redundancy of photoperiodic and temperature mechanisms? The fact is that the photoperiodic mechanism has never been unambiguously demonstrated at all! Diapausing pupae will never eclose unless chilled for a number of weeks. Although we know that temperature treatment has no effect on adult pigmentation when the pupa is in deep diapause, what if diapause intensity gradually decreases, allowing for increasing sensitivity to environmental cues? This is precisely the clinical picture of diapause being advanced by Tauber and Tauber at Cornell (see their recent review in *Annual Review of Entomology*). We are currently testing this hypothesis by monitoring the day-to-day metabolism of diapausing *napi* pupae and subjecting them to various temperature regimes when they begin to wake up.

Underlying whatever proximate, i.e. physiological, answers to our "why" question are genetic answers, and these reflect a history of natural selection. The ecological basis of selection — the adaptive value — is the ultimate level of causality. Ward Watt at Stanford, formerly at Yale, followed up on Leigh and Smith's work and demonstrated the efficacy of seasonal phenotypes in body-temperature regulation of the Orange Sulphur, *Colias eurytheme*. Our work points in the same direction for *Pieris napi*. One striking aspect is the consistent sexual difference in *napi* — in all populations and in both seasonal phenotypes, males are more heavily marked than females. Field studies suggest that this confers an adaptive advantage in that the darkest males can become active at lower temperatures in the morning, giving them first crack at newly-emerged virgin females. (*Pieris napi*, like most butterflies, has a diel periodicity of emergence, with most eclosions occurring shortly after sunrise even at low temperatures.)

I hope the preceding remarks have given you some feel for the directions in which polyphenism studies are going. But I'd like to wind up with some comments on their biosystematic implications for *Pieris napi*, because I have a petition in to the International Commission on Zoological Nomenclature that is apt to raise some eyebrows when it appears in the *Bulletin*. I might add, with no snobbery intended, that I commit taxonomy only when dragged into it kicking and screaming by the biology.

I said several minutes ago that everyone "knew" that Californian *napi* had two seasonal phenotypes, *venosa* and *castoria*. Last year Bob Langston suggested at these meetings that they might be two different species; our work dispels that notion. Clearly, they are seasonal phenotypes produced by the same genome responding to different environmental cues. The problem — as Langston first pointed out to me — is that what we have been calling "castoria" isn't what Reakirt described as *castoria* in 1867! Here is Reakirt's O.D.:

Size and form of *Pieris oleracea*, Harris.

*Male*, upper side pure white, inner half of costa of primaries, and base of both wings, strewn with a few dark atoms; a rounded black spot in the medio-superior interspace of the fore wings . . . no other markings; fringes white, expanse 2-2.12 inches.



Underneath immaculate white; a faint yellowish tinge on the apex of the primaries, and along the costa of the secondaries.

Body black, with whitish hairs below; antennae black, with incomplete white annulations interrupted above. Club yellowish, or yellowish brown at tip.

*Hab.* — California. Coll. Tryon Reakirt.

Herman Strecker, in 1877, was the first to consider *castoria* as the second brood phenotype of double-brooded California *napi*; in this he was followed by W. H. Edwards in his (1881) revision of the *napi* group, and by most authors since. Yet very, very few specimens collected in the wild from double-brooded populations come near to this description. Almost all of them have some dark scaling on the veins beneath, at the apex of the fore- or hindwing above, or whatever. When it turned out that the so-called type of *castoria* at the Field Museum was a pseudotype (in fact it is a typical second brood specimen from a coastal population, matching Strecker's sense but not Reakirt's description of *castoria*!), it was exceedingly difficult to find prospective neotypes that matched the O.D. Now, as it happens the O.D. matches *perfectly* the normal phenotype produced by non-diapause pupae of interior, single-brooded California *napi*. Confused? Let's backtrack a bit.

Our experiments concur with field data in suggesting that there are two subspecies of *napi* in central California. Subspecies *venosa* is found on and near the coast, in places subject to summer fog; it is best developed from San Francisco south. It is at least partially double-brooded, with the two seasonal phenotypes. In the hot, dry, fogless Inner Coast Range and on the west slope of the Sierra Nevada occurs a single-brooded subspecies, showing only a spring phenotype in nature. The oldest available name for this population is usually considered to be *microstriata* Comstock, 1925. The spring phenotype of *microstriata* is lighter than that of *venosa* reared under identical conditions. When diapause is artificially prevented, *microstriata* will make a summer phenotype in the lab, and it matches perfectly Reakirt's O.D. of *castoria* — being lighter than the summer phenotype produced by *venosa*. Recently we have found a couple of places, in cold canyons, where *microstriata* is naturally double-brooded and makes a summer phenotype just like our lab one. This raises an uncomfortable taxonomic specter.

Suppose it could be shown that what Reakirt had was not a second brood coastal specimen at all, as everyone has now assumed for 99 years, but one of the very rare interior ones? Then the oldest valid name for the inland subspecies would be *castoria*, and we would be obliged to use it; the familiar usage for the second-brood phenotype of *venosa* would be scrapped, and the name *microstriata* would sink into oblivion.

And everyone would hate *me*.

Where *did* Reakirt's type come from? "California," says the O.D. But F. Martin Brown has shown that Reakirt never collected in California; he got his stuff from Lorquin. Where did Lorquin get it? Unfortunately there is still too little evidence at hand to reconstruct his itineraries very well; we know he collected in both *venosa* and *microstriata* country, though. I was wrestling with this when I noticed that, in the same paper as he describes *castoria*, Reakirt also describes *Pieris yreka*. Now, this is rather an infamous description since it can only apply to *rapae* — and the type, at the Field Museum, is a spring *rapae* — thus implying that *rapae* was in California before 1867, which is inconsistent with the idea of a single introduction in southern Canada about 1860 — but we wander. The point is that, although the type locality of *yreka* is not specified, it's a good bet it was the town of Yreka. I checked this out in that invaluable resource, Gudde's *California Place Names*, and found that Yreka was named in 1852. Well, if Yreka was the type locality of *Pieris yreka*, was Castoria the type locality of *Pieris castoria*? Here's what Gudde says about the town of Castoria: Castoria = French Camp, San Joaquin County . . . known as Castoria (Latin for *beaver*) from 1850-59 . . . formerly the headquarters of French beaver trappers on the San Joaquin River . . . ! Could Lorquin, a Francophone, stay away from such a place?

Alas, there are to be no topotypes. 125 years ago French Camp was in a maze of riparian woodland and marshland; today it has been thoroughly civilized, and there are no *napi* there. In fact, there are no *napi* currently known *anywhere* on the floor of the Great Central Valley. Still, on climatic and biogeographic grounds we can infer that any population at French Camp must have been of the interior subspecies — thus our taxonomic nightmare becomes real.

So I have asked the Commission to do something very odd. I have asked them to suppress the name *castoria* under the

Plenary Powers for synonymy but not homonymy. If they do, it will be unavailable for use at the subspecific (or specific) level, but can continue to be used informally, as a seasonal phenotype name, the way it is now and has been for 99 years. It was necessary to take this roundabout route because *microstriata* hasn't been used often enough in the literature to qualify for conservation under Article 79 — yet common sense demands that nearly a century of usage of *castoria* not be upset just because some dingbat at Davis finds that there's more to *napi* than meets the eye. Like I said, I do taxonomy only under compulsion.

Now do you see why I'm not here?